AN EXPERIMENTAL STUDY ON THE ROLE OF THE RETICULOENDOTHELIAL SYSTEM IN HOST RESISTANCE AGAINST TUMOR ; WITH SPECIAL REFERENCE TO THE SPLEEN

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AN EXPERIMENTAL STUDY ON THE ROLE OF THE RETICULOENDOTHELIAL SYSTEM IN HOST RESISTANCE AGAINST TUMOR ;

WITH SPECIAL REFERENCE TO THE SPLEEN

By

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I. INTRODUCTION.

It is generally accepted by surgeons that cancer grows in a steady and irrevocable manner despite therapies of almost all kinds, suggesting its absolute potentiality of growth. Deliberate observations of individual cases, however, give an impression to deny this conception, that is, course of tumor is not always one-way path, but considerably irregular[19]. Sometimes slow and mild growth of tumor is suddenly taken place by rapid overwhelming growth which leads to death[25,29]. There can be seen many reports of cases in which tumor grew very slowly without any particular treatment, or cases which showed favorable prognosis for many years after mere a palliative surgery and not a few of these remained symptomless for decades until late recurrence or late metastasis[25]. On the contrary, it is a common experience of surgeons that early recurrence or metastasis can be observed even in cases of adequately performed radical operation.

On the other hand, there are many reports on the observations of spontaneous regression of once established tumor[16], regression of metastatic tumor following excision of primary lesion[17,42,47,56,64] and histiocytic or lymphatic reaction around some tumors or in the regional lymph nodes[8,60]. Moreover, it is pointed out that despite the existence of numerous tumor cells floating in the blood stream, majority of the cells do not develop to establish metastases[36,44,49] and that stress factors, such as irradiation or steroid hormone, enhance growth of both primary and secondary tumor[13,15,43].

All these findings are accepted to suggest strongly that tumor growth is not always permitted by the hosts, but they attempt to some extent certain resistance against it. At this point, there is an attempt to grasp tumor growth under a concept of 'host-tumor relationship', which is gradually occupying large area of cancer investigation in recent years. Under such a concept, experiments are carried out by many researchers to investigate tumors biologically and to express malignancy or growth potentiality quantitatively. GREENE (1951)[23,24] tried quantitative expression of tumor autonomy or malignancy by transplanting human cancer into the anterior chamber of the eye or the brain of rodent

animals and the buccal sack of hamster, which was examined later by TOWBIN (1951)[63] who intended clinical application of this method. This was, however, impossible owing to its very low transplantability. In the similar aim, TOOLAN (1953)[62] made experiments to transfer human cancer into animals previously treated with cortisone or irradiation. In 1958, AMOS[4] and his co-workers succeeded in drawing growth curve of ascites carcinoma in mice by the use of diffusion chamber, and established a new approach to the clinical application.

On the other hand, there are also numerous investigations to explore the mechanism of host defence against cancer[39,40,41,54,68]. It is easily accepted that the reticuloendothelial system, which plays an important role in various defence of organism, should participate similarly in antitumoral defence to large extent, as in infections[28,29,40,46]. The spleen that involves one third of the reticuloendothelial system in the whole body has attracted interest of many investigators as a source of antitumoral resistance. Particularly, very rare incidence of both primary and secondary tumors in this organ[12], almost constant failure of tumor implantation into parenchyma of the organ[7] and conspicuous splenic enlargement in the course of the most of experimental tumors[12,33,40,46] have all been regarded as strong basis to presume defence mechanism against tumor in the spleen. Although there are many reports to approve of this presumption, very little has been explored concerning the mechanism of this defence, which is presumably due to the fact that the physiology of the spleen is not yet clearly understood, that there are many differences in the problem of immunity between human cancer and experimental tumor and moreover the fact that many complicated problems are contained in analysis of antigenicity of tumor cell, even in occasion in which existence of tumor immunity is ascertained[20,66].

The author of the present experiment intended to study the antitumoral effect of the spleen and to explore the relationship between this defence and reticuloendothelial function using ascites hepatoma AH 130 in rats.

Ⅱ. MATERIALS AND METHODS.

1. Materials.

Animals : 702 random-bred rats of Gifu-strain, weighing 80 to 105 g, were fed by mixed diet and used in the present experiment.

Tumor : Ascites hepatoma AH 130 A1106 was used since 428 th generation which was maintained weekly in the peritoneal cavity of the rats.

2. Methods.

a. Method of inoculation. Inoculations were performed constantly under aseptic condition.

i. Intraperitoneal inoculation.

Tumor cells of 7 day intraperitoneal growth were aspirated by peritoneal tap and cell count was performed in hemocytometer. Six million of tumor cells was inoculated into the peritoneal cavity.

ii. Subcutaneous inoculation.

Similarly aspirated tumor cells of 1200×10^1 were inoculated subcutaneously in the right gluteal region previously shaved.

b. Observation of tumor growth.

i. Intraperitoneal growth.

Tumor was taken in 100 per cent of animals. Appearance of tumor growth was determined by survival days.

ii. Subcutaneous growth.

In subcutaneous inoculation, although accompanied by considerable fluctuation, "no take" was frequently observed, which is accepted to be partly due to condition of tumor cells, and sometimes conspicuously retarded growth was seen even in cases of "take", most of which showed spontaneous regression of tumor sooner or later. Accordingly, both cases in which tumor development was not observed and cases in which the maximum diameter of tumor did not reach 1 cm within a week were excluded from experiment being regarded as "no take", which was observed in 22.0 per cent on the average. Since subcutaneous tumor does not always enlarge semispherically, the maximum diameter was measured by calipers every other day.

c. Preparation of spleen homogenate.

Spleen homogenate was prepared under sterile condition, and unless otherwise mentioned the homogenate was prepared from 8 day aged tumor-bearing animals. Animals were slaughtered by bleeding from the inferior vena cava under ether anesthesia. About 300 cc of saline was transfused into the left ventricle and let flow out from the inferior vena cava in order to get rid of blood from the body as possible. The spleen was then taken out and weighed on torsion balance. The organ was minced by the scissors and well gronud in a glass homogenizer, filtrated through 2 sheets of gauze and diluted with saline. Since long persisting induration was observed after subcutaneous injection of homogenate of high concentration, homogenate was diluted to 20 per cent, which was the maximum concentration that did not cause induration. The homogenate thus prepared was preserved in 4°C, being added with merzonin in 0.01 per cent.

d. Injection of spleen homogenate.

The spleen homogenate of 0.5 cc was injected subcutaneously in the inguinal region once a day from the day of intraperitoneal inoculation for 3 days successively, while in subcutaneous growth the injection was initiated from 11 days after inoculation 3 times every other day.

e. Histological examinations of spleen and tumors.

Spleen and tumor which showed a tendency of regression were removed and fixed in 10 per cent neutral formalin and stained with hematoxylin and eosin for histological examinations.

f. Electrophoretic studies of spleen homogenate.

The homogenate was centrifuged at a frequency of 1000 r. p. m. for 5 minutes and the protein content of the supernatant was adjusted to 7.0 g/dl with saline and provided for electrophoretic material. Since considerable amount of the material remained in original point when veronal buffer was used, which is usually prefered in electrophoresis of serum, borate buffer of Clark and Lubs was adopted[1,31] Electrophoresis was performed for 7 cm under constant current of 5 mA with borate buffer of pH 8.6[37] After electrophoresis, the filter paper was dried at 120°C for 20 minutes, stained with brom-phenol-blue solution for 20 minutes and bleached with acetic acid of 1.5 per cent, which was then dried in

room temperature and made semitransparent with paraffin for densitometry[37].

g. Examinations of reticuloendothelial function.

Weight of spleen and liver which are deemed to be the most important organ of reticuloendothelial system[40,45], splenic uptake of colloidal radiogold (^{198}Au-colloid)[55] and congo-red index were examined[2,67,68]. It is widely recognized that reticuloendothelial function is subtly affected by various physical conditions, therefore all these examinations were performed in the fasting state[40,67].

i. Weighing of spleen and liver.

Animals were slaughtered by bleeding under deep anesthesia with ether. The spleen and liver were extirpated and weighed on torsion balance, after blood on their surface removed with filter paper. Weight of these organs was expressed in the terms of spleen index and liver index, which were calculated as percentage to the body weight[38,45].

ii. Splenic uptake of colloidal radiogold.

Colloid of radioactive gold was provided from Japanese Corporation of Radioactive Isotope. Diameter of the colloid used here was 20 to 25 mμ. Estimation was performed after the method of STERN and DUWELIUS[55], except that the colloid was administered by intravenous injection since by intraperitoneal injection, as described by them, considerable amount of radioactive colloid remained in the peritoneal cavity even 24 hours afterwards, which caused unnegligible error of data. Colloidal radiogold of 0.2μC per g body weight was injected into the tail vein. Twenty-four hours later, the animals were slaughtered with deep anesthesia of ether and abdominal cavity was rinsed with 20 cc of saline for 3 times. The spleen was extirpated, minced and ground into homogenous suspension in saline. Radioactivity of the homogenate of 1 cc was counted in a well-type scintillation counter. Neither the blood of 1 cc nor 1 cc of saline used for the rinse of the abdominal cavity proved particular radioactivity.

iii. Congo-red test.

ADLER-REIMANN-SUGIYAMA's method[2,67,68] was followed, except that the amount of blood material was lessened and serum was diluted with saline for spectrophotometry, since the animals could not bear being drawn large amount of blood as described in their method[2,68]. Congo-red saline solution of 0.1 per cent was injected into the tail vein in the proportion of 1 cc per 100 g body weight. Four and 60 minutes after the injection, accurately 0.4 cc of blood was drawn from the femoral vein with tuberculin syringe containing 0.1 cc of 3 8 per cent soduim citricum. Saline of 1.5 cc was added to the blood drawn and centrifuged at 2000 r. p. m. for 10 minutes. The supernatant was separated and its concentration of congo-red was estimated with spectrophotomer of Coleman through the filter of 510 mμ[48]. Congo-red index was calculated from following formula ;

$$\text{Congo-red index} = \frac{\text{Concentration of congo-red in serum of 4 minutes after the injection}}{\text{Concentration of congo-red in serum of 60 minutes after the injection}}$$

h. Incubation of tumor cells with spleen homogenate.

One cc of intraperitoneal 7 day growth was incubated at 37°C for 1 hour with 0.3 cc of spleen homogenate, 0.2 cc of which was subcutaneously inoculated.

i. Reticuloendothelial blockade with india ink.

Commercial india ink was filtrated twice through 2 sheets of filter paper, 100 cc of which was dialysed twice against distilled water of about 4000 cc for 24 hours through

cellophane membrane[5,37,40,67]. Dialysed india ink was diluted with saline to 15 per cent and sterilized at 73°C for 2 hours a day for 3 days. Reticuloendothelial blockade was performed by the injection of india ink of 0.5 cc per 100 g of body weight from the tail vein at least for 15 days successively[5,32].

Ⅲ. RESULTS.

1. Transplantability and survival days in intraperitoneal inoculation.

Transplantability of ascites hepatoma AH 130 in the peritoneal cavity was observed to be 100 per cent ; no case of "no take" and spontaneous regression was observed. All the animals given intraperitoneal inoculation died within 8 to 14 days. Average survival days of 19 animals was 11.3 days (Tab. 1).

2. Inhibitory effect of spleen homogenate from tumor-bearing animals on intraperitoneal growth.

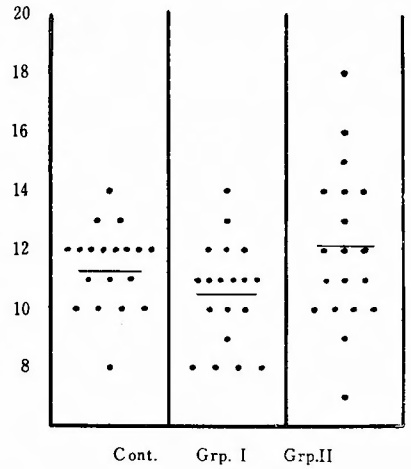
Fifty-seven rats given intraperitoneal inoculation were divided into 3 groups, that is, a group of control, a group for injection of spleen homogenate from normal animals and a group for injection of spleen homogenate from tumor-bearing animals. All the animals of three groups died and any difference in survival days could not be observed compared with control. Namely, survival days of control animals ranged from 8 to 14 days, 11.3 days on the average. Survival days of the animals treated with spleen homogenate from normal animals ranged from 8 to 14 days, 10.5 days on the average. The animals trea-

Tab. 1. Inhibitory Effect of Spleen Homogenate from Tumor-bearing Animals on Intraperitoneal Growth.

	Injection	No. of Inoculation	No. of "No Take"	No. of "Take"	No. of Regression	Survival Time in Days*
Exp. I	Control	9	0	9	0	11.3 (8-14)
	Spleen Homogenate from Normal Animal	9	0	9	0	10.3 (8-14)
	Spleen Homogenate from Tumor-bearing Animal	9	0	9	0	12.3 (10-18)
Exp. II	Control	10	0	10	0	11.5 (10-13)
	Spleen Homogenate from Normal Animal	10	0	10	0	10.6 (8-12)
	Spleen Homogenate from Tumor-bearing Animal	10	0	10	0	10.8 (7-14)
Total	Control	19	0	19	0	11.3 (8-14)
	Spleen Homogenate from Normal Animal	19	0	19	0	10.5 (8-14)
	Spleen Homogenate from Tumor-bearing Animal	19	0	19	0	12.1 (7-18)

* Average of Animals died of Tumor.

Fig. 1. Survival Days of Animals with Intraperitoneal Growth.



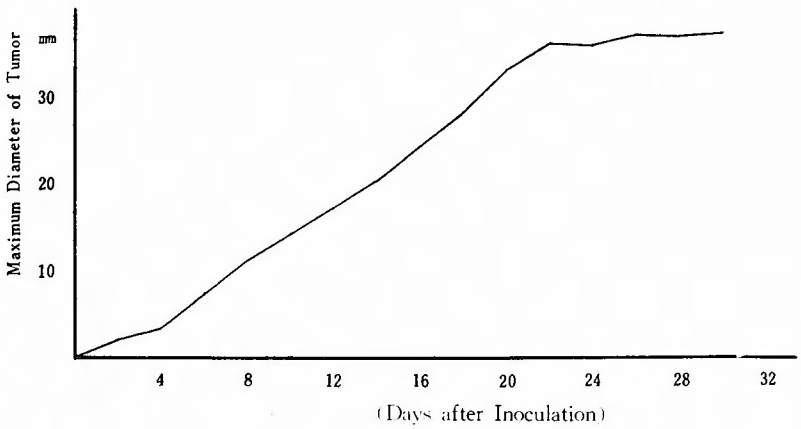
Grp. I Received transfer of spleen homogenate from normal animals.
Grp. II Received transfer of spleen homogenate from tumor-bearing animals.

ted with spleen homogenate from tumor-bearing rats died within 7 to 18 days, 12.1 days on the average, revealing a slight prolongation of survival days in 1 case (Tab. 1, Fig. 1). Accordingly, it was assumed that potentiality of tumor growth was so intense in the abdominal cavity as the original site of the tumor that the spleen homogenate revealed no inhibitory effect on tumor growth.

3. Transplantability, survival days and frequency of spontaneous regression in subcutaneous inoculation.

In subcutaneous inoculation of ascites hepatoma, "no take" was observed frequently in 0 to 60 per cent, 22.0 per cent on the average, with considerable fluctuation, which was assumed to be influenced partly by condition of tumor cells. Subcutaneous tumors became palpable towards 4 days after the inoculation, its maximum diameter reached about 10 mm towards 8 days after the inoculation and about 30 mm towards 20 days after the inoculation. The tumors enlarged thereafter on gradually and the animals died of cachexy due to tumor growth (Fig. 2). Survival days ranged from 13 to 26 days, 19.8 days on the average of 50 cases (Tab. 2, Fig. 3), It was observed sometimes that

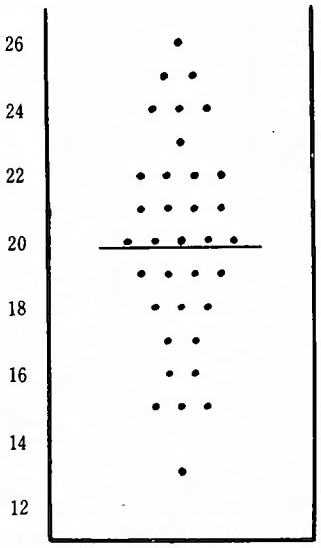
Fig. 2. Regular Growth of Subcutaneous Tumor. (Average of 50 animals)



Tab. 2. Regular Development of Subcutaneous Transplants.

	No. of Inoculation	No. of "No Take" (%)	No. of "Take"	No. of Regression (%)	Average Survival Days of Animals Died of Tumor
Exp. I	15	4 (26.6)	11	2 (18.2)	19.7 (15–25)
Exp. II	20	4 (20.0)	16	2 (12.5)	17.8 (13–26)
Exp. III	15	3 (13.3)	12	0 (0)	19.3 (15–25)
Total	50	11 (22.0)	39	4 (10.3)	19.8 (13–26)

Fig. 3. Survival Days of Regular Subcutaneous Growth.



tumors ceased their growth when their maximum diameter reached 20 mm and gradually tended to spontaneous regression in 10.3 per cent (Tab. 2).

4. Reticuloendothelial function during the course of subcutaneous growth.

As an examination of reticuloendothelial function, congo-red test is widely employed. Besides this, fluctuation of spleen and liver weight and splenic uptake of colloidal radio-gold were estimated as indications of reticuloendothelial function.

a. Spleen index.

As subcutaneous tumor grew larger, conspicuous enlargement of the spleen was observed having its peak 8 days after inoculation to be 0.63 on the average, almost twice as much compared with the average of normal of 0 34, which then decreased as the tumor grew huge and restored to normal 24 days after inoculation. Spleen index showed thereafter further tendency of decrease to be 0 28 on the average 28 days after inoculation, which is smaller than normal (Tab. 3, Fig. 4, a).

From these findings it is presumed that in the early stage of tumor growth the spleen endeavours to promote resistance of organism showing marked enlargement, which, however, cannot surpass overwhelming growth of tumor and finally becomes atrophied

being exhausted out.

b. Liver index.

Fluctuation of liver index behaved roughly in parallel with that of the spleen, showing its peak 8 days after inoculation to be 4.6 on the average and decreased on thereafter to be 3.3 on the average 24 days after inoculation which is equal to normal (Tab. 3, Fig. 4, b).

c. Splenic uptake of colloidal radiogold.

Fluctuation in the splenic uptake of colloidal radiogold was observed to be considerably slight. The uptake was heightened to 0.48 per cent on the average 8 days after inoculation and 0.47 per cent on the average 12 days after inoculation, which then de-

Tab. 3. Fluctuation of Reticuloendothelial Function during the Course of Subcutaneous Tumor Growth.

	Cont.	Days after Inoculation							
		2	4	8	12	16	20	24	28
No. of animals	20	16	19	20	19	15	17	13	18
Spleen index	0.34 (0.10-0.74)	0.37 (0.20-0.67)	0.53 (0.21-0.92)	0.63 (0.27-1.65)	0.50 (0.27-1.01)	0.48 (0.29-0.90)	0.42 (0.11-0.96)	0.38 (0.18-0.80)	0.28 (0.15-0.63)
Liver index	3.8 (3.2-5.6)	3.9 (3.4-4.6)	4.1 (3.8-4.6)	4.6 (3.7-5.2)	3.9 (3.3-4.5)	3.6 (3.0-4.5)	3.6 (3.0-4.2)	3.3 (2.8-3.7)	3.1 (2.6-3.6)
Splenic uptake of ^{198}Au-colloid (%)	0.38 (0.21-0.64)	0.39 (0.28-0.81)	0.39 (0.31-0.53)	0.48 (0.24-0.87)	0.47 (0.24-0.77)	0.35 (0.25-0.52)	0.36 (0.22-0.89)	0.35 (0.12-0.71)	0.23 (0.11-0.33)
Congo-red index	1.78 (1,11-2.51)	2.29 (1.28-3.02)	2.07 (1.29-3.05)	2.76 (2.48-3.44)	2.37 (1.84-3.33)	1.96 (1.20-2.97)	1.68 (1.13-2.55)	1.43 (1.11-2.11)	1.37 (1.00-2.05)

Fig. 4. Fluctuation of Reticuloendothelial Function in Subcutaneous Growth. a. Spleen Index.

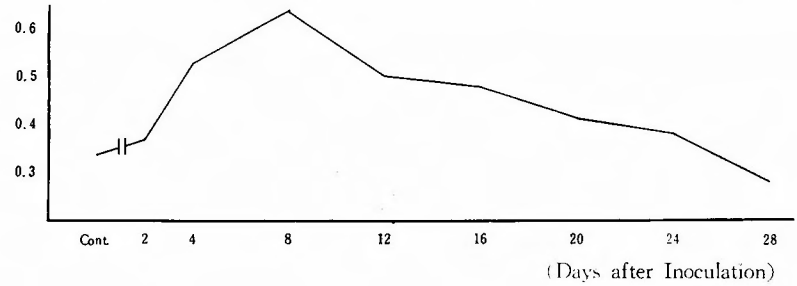


Fig. 4 Fluctuation of Reticuloendothelial Function in Subcutaneous Growth. b. Liver Index.

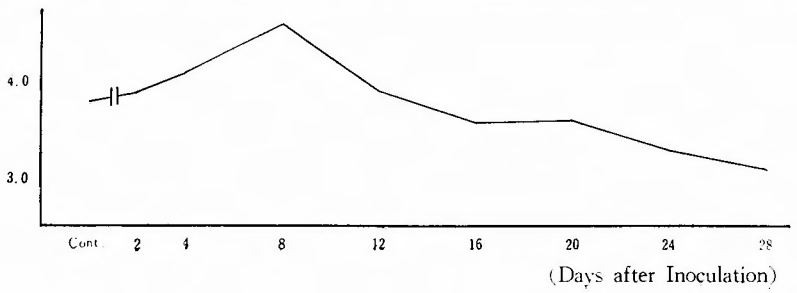


Fig. 4. Fluctuation of Reticuloendothelial Function in Subcutaneous Growth.
c. Splenic Uptake of ^{198}Au-colloid.

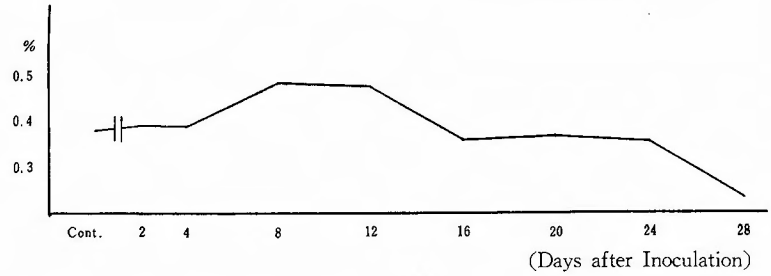
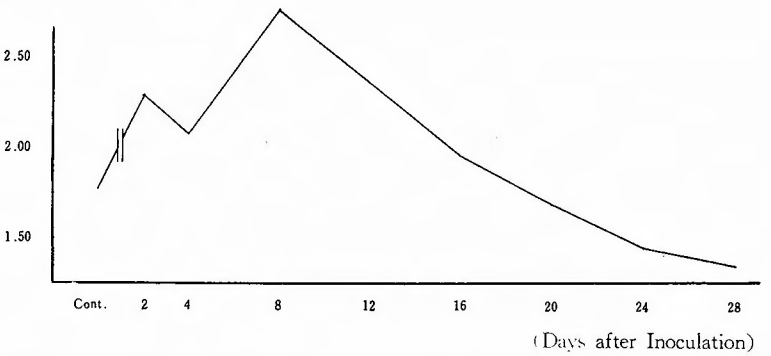


Fig. 4. Fluctuation of Reticuloendothelial Function in Subcutaneous Growth.
d. Congo-red Index.



creased gradually to 0.23 per cent on the average 28 days after inoculation which is smaller than normal of 0.38 per cent (Tab. 3, Fig. 4, c).

d. Congo-red index.

Congo-red index of 20 control animals was 1.78 on the average. The index was observed conspicuously to increase 8 days after inoculation to be 2.76 on the average, which then decreased on as tumor grew, becoming 1.68, already 20 days after inoculation, which was below normal, and decreasing further to be 1.37, 28 days after inoculation (Tab. 3, Fig. 4, d).

From all these results of examinations of reticuloendothelial function during the course of tumor development, some correlation was recognized between reticuloendothelial function and subcutaneous tumor development of ascites hepatoma AH 130, that is, reticuloendothelial function is heightened temporarily having its peak 8 days after inoculation. Accordingly, it is obviously assumed that until this stadium the reticuloendothelial system endeavours to resist against tumor growth as a part of defence mechanism of organism, which is, however, defeated by endless and overwhelming growth of tumor and it comes to be entirely exhausted out.

5. Histological and electrophoretic studies of the enlarged spleen of tumor-bearing animals.

The most outstanding features of histological finding in the most conspicuously enlarged spleen of 8 day aged tumor-bearing animals, when the reticuloendothelial function

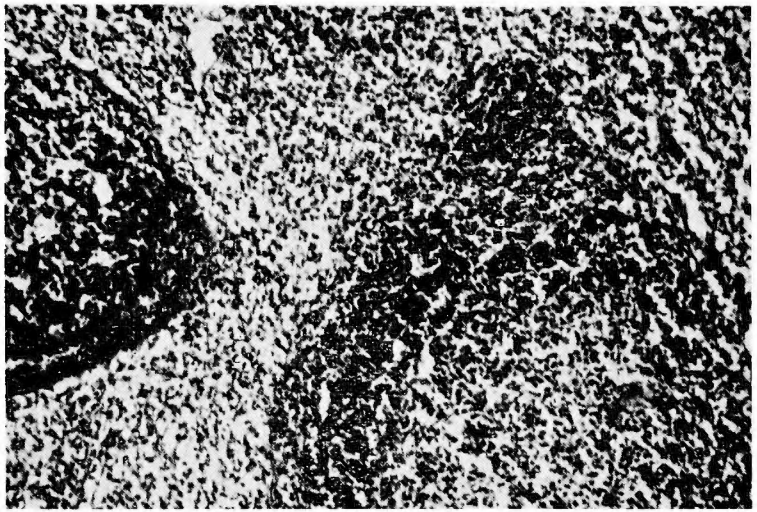


Fig. 11. Spleen of normal rat. (H-E) (×100)

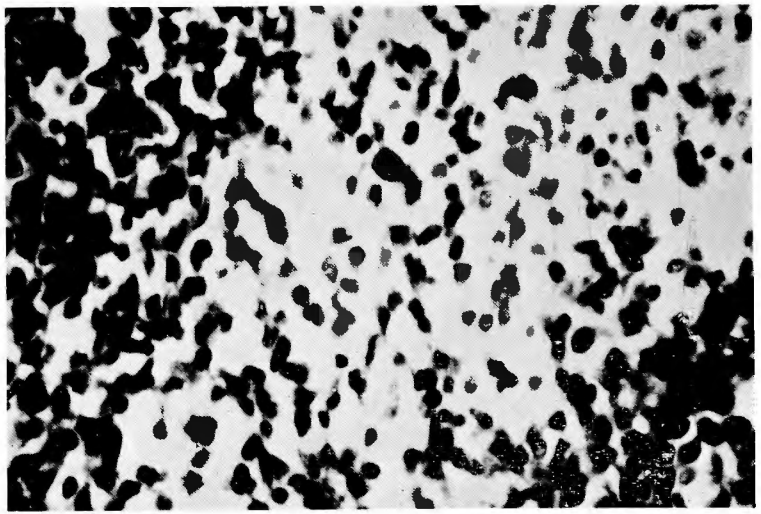


Fig. 12. Spleen of normal rat. (H-E) (×400)

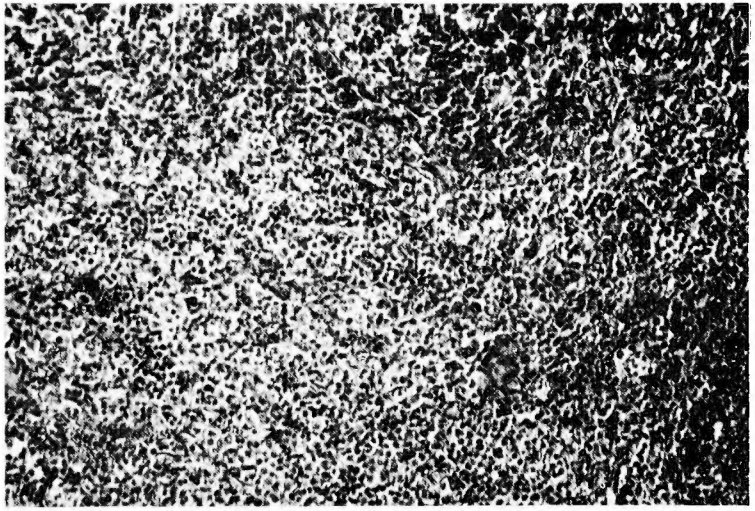


Fig. 13. Enlarged spleen of tumor-bearing rat. (H-E) (×100)

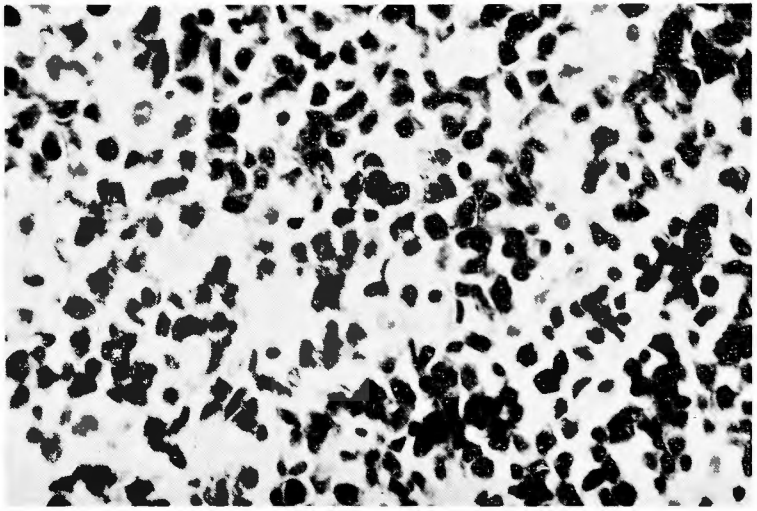


Fig. 14. Enlarged spleen of tumor-bearing rat. (H-E) (×400)

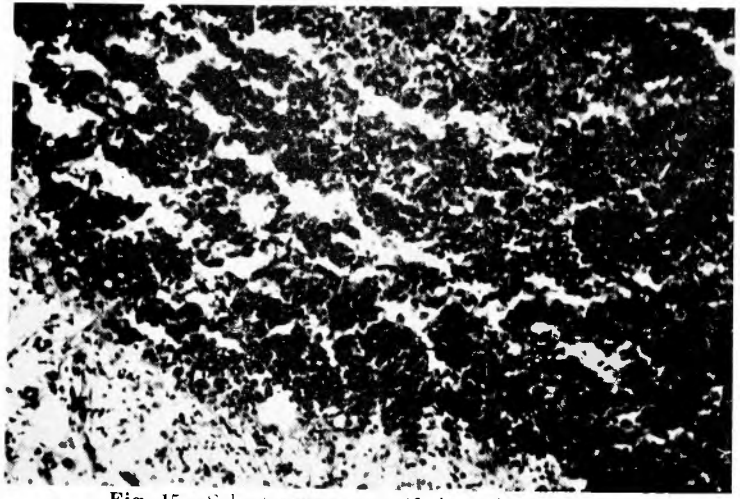


Fig. 15. Subcutaneous tumor 10 days after inoculation. (H-E) (×100)

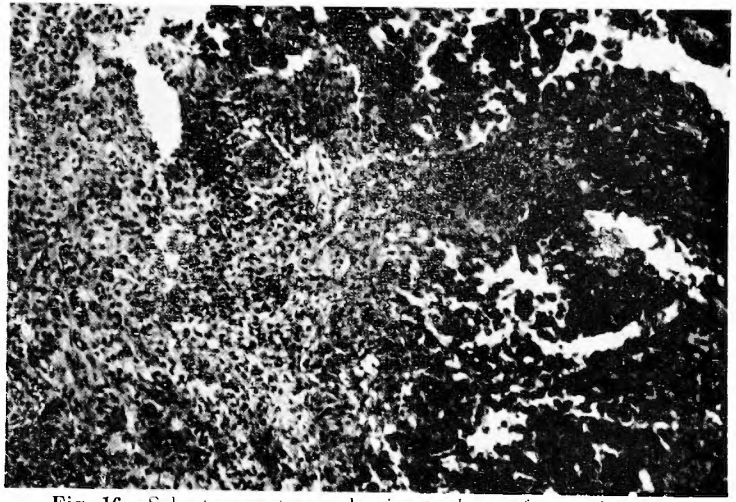


Fig. 16. Subcutaneous tumor showing tendency of regression, 24 days after inoculation. (H-E) (×100)

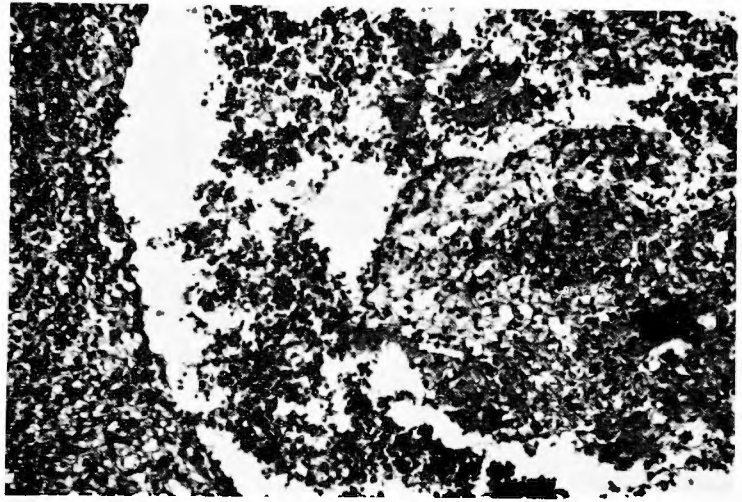


Fig. 17. Subcutaneous tumor showing tendency of regression, 34 days after inoculation. (H-E) ×100)

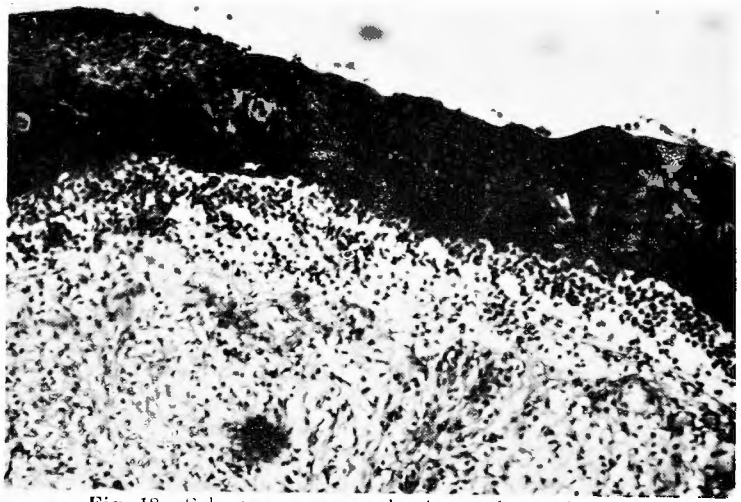


Fig. 18. Subcutaneous tumor showing tendency of regression, 42 days after inoculation. (H-E) (×100)

was in its most heightened state, were hypertrophy and hyperplasia of splenic pulp caused by impressive increase in plasma cells and histiocyts, consequently causing marked increase in density of cells in the pulp. The cell density in the pulp decreased thereafter in parallel with decrease of spleen index. Hypertrophy of lymph follicle and appearance of giant cells as reported by Roffo and others were not observed in the present experiment (Fig. 11, 12, 13, 14).

Electrophoretic studies of the enlarged spleen of this stadium revealed a pattern of single marked peak corresponding to γ-globulin. Although some correlation between amount of this fraction and marked increase in plasma cells was infered, this fraction of every stadium of tumor development maintained constant value regardless of the fluctuation of plasma cells in the spleen (Tab. 4).

6. Inhibitory effect of spleen homogenate from tumor-bearing animals on subcutaneous growth.

a. Inhibitory effect of spleen homogenate from animals bearing 8-day-aged subcutaneous tumor.

Five experiments were performed repeatedly and single experiment contained 30 to 36 animals, which were divided into 3 groups, that is, control animals, animals for injection of spleen homogenate from normal animals and those for injection of spleen homogenate from tumor-bearing animals (Tab. 5). Injection of spleen homogenate was initiated from 11 days after subcutaneous inoculation. In the group received transfer of spleen homogenate from tumor-bearing animals, 16 "no takes" were observed in 54 inoculations of 5 experiments, and out of remaining 38 animals, tumor regression was observed in 29 cases. In the control group, 10 cases of "no take" and 10 cases of tumor regression were observed in 54 animals. In the group received transfer of spleen homogenate from normal animals, 10 cases of "no take" and 9 cases of tumor regression were observed in 54 animals. Outstanding difference in tumor regression was still realized between the group received transfer of spleen homogenate from tumor-bearing animals and two other groups, even if the wide fluctuation of transplantability observed at inoculation was taken into consideration. In cases in which tumor regression was observed, subcutaneous tumor disappeared entirely within 45 days and the animals survived on. Survival days of animals died of tumor despite the transfer of spleen homogenate from tumor-bearing animals ranged from 15 to 27 days, 22.1 days on the average, having little difference compared with two other groups of control and animals received transfer of spleen homogenate from normal animals, that is, 13 to 28 days, 20.2 days on the average and 14 to

Tab. 4. Fluctuation of Content of a Fraction corresponding to γ-Globulin in Spleen Homogenate from Tumor-bearing Animals.

	Control	Days after Inoculation 4	8	16	20	28
No. of Animals	20	10	18	12	22	12
Content of Fraction corresponding to γ-Globulin (%)	73.9 (52.6-86.1)	74.1 (56.8-84.0)	76.3 (61.1-87.5)	74.6 (62.8-80.4)	73.4 (60.5-87.9)	70.9 (62.4-88.0)

Tab. 5. Inhibitory Effect of Spleen Homogenate from Tumor-bearing Animals on Subcutaneous Growth.

	Injection	No. of Inoculation	No. of "No Take" (%)	No. of "Take"	No. of Regression	No. of Death	Rate of Regression (%)
	Control	12	3(25.0)	9	0	9	0
I	SH. f. NA.*	12	3(25.0)	9	1	8	11.1
	SH. f. TA.**	12	1(8.3)	11	9	2	81.8
	Control	10	4(40.0)	6	1	5	16.7
II	SH. f. NA.	10	3(30.0)	7	2	5	28.6
	SH. f. TA.	10	6(60.0)	4	3	1	75.0
	Control	10	2(20.0)	8	3	5	37.5
III	SH. f. NA.	10	2(20.0)	8	2	6	25.0
	SH. f. TA.	10	4(40.0)	6	5	1	83.3
	Control	10	1(10.0)	9	2	7	22.2
IV	SH. f. NA.	10	0(0)	10	2	8	20.0
	SH. f. TA.	10	2(20.0)	8	6	2	75.0
	Control	12	0(0)	12	4	8	33.3
V	SH. f. NA.	12	2(16.6)	10	2	8	20.0
	SH. f. TA.	12	3(25.0)	9	6	3	66.7
	Control	54	10(18.5)	44	10	34	22.7
Total	SH. f. NA.	54	10(18.5)	44	9	35	20.4
	SH. f. TA.	54	16(29.6)	38	29	9	76.3

* Spleen homogenate from normal animals.
** Spleen homogenate from tumor-bearing animals.

29 days, 19.9 days on the average, respectively (Tab. 5, Fig. 5).

b. Inhibitory effect of spleen homogenate from animals bearing 20-day-aged subcutaneous tumor.

It was ascertained that the spleen homogenate shows remarkable inhibitory effect upon subcutaneous growth, when the homogenate is prepared from animals bearing subcutaneous tumor of 8 day growth when reticuloendothelial function is in its utmost activity. In order to explore the existence of correlation between this inhibitory effect and reticuloendothelial function of the animals from which spleen homogenate is prepared, similar experiments on inhibitory effect were made using spleen homogenate which was prepared from 20 day aged tumor-bearing animals when their reticuloendothelial function is depressed.

Fifty animals were divided into 2 groups of control animals and those for injection of spleen homogenate from 20 day aged tumor-bearing animals, and the injection of spleen

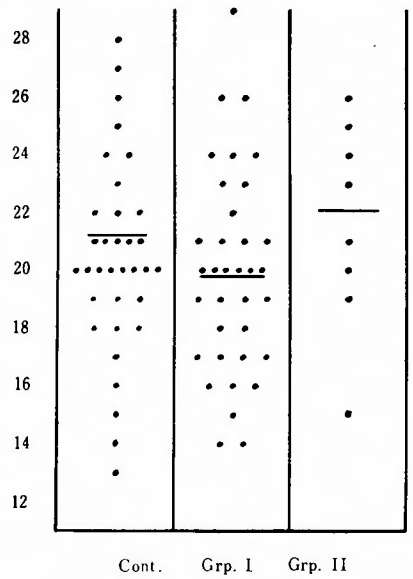
homogenate was initiated from 11 days after the inoculation.

In control group, 3 cases of "no take" were observed in 23 inoculations and out of remaining 20 animals tumor regression was observed in one case. On the other hand in the group received transfer of spleen homogenate from 20 day aged tumor-bearing animals, 5 cases of "no take" out of 27 inoculations and 4 tumor regressions out of remaining 22 animals were observed, the rate of regression being 5.0 per cent and 18.2 per cent, which

Tab. 6. Inhibitory Effect of Spleen Homogenate from 20-day-aged Tumor-bearing Animals on Subcutaneous Growth.

	Injection	No. of Inoculation	No. of "No Take" (%)	No. of "Take"	No. of Regression (%)	Survival Days
Exp. I	Control	13	1(7.7)	12	1(8.3)	22.7 (16–25)
	Spleen Homogenate from Tumor-bearing Animals	15	2(13.3)	13	3(23.0)	19.7 (15–26)
Exp. II	Control	10	2(20.0)	8	0 (0)	20.5 (16–27)
	Spleen Homogenate from Tumor-bearing Animals	12	3(25.0)	9	1(11.1)	20.3 (16–26)
Total	Control	23	3(13.1)	20	1(5.0)	21.8 (16–27)
	Spleen Homogenate from Tumor-bearing Animals	27	5(18.5)	22	4(18.2)	19.4 (15–26)

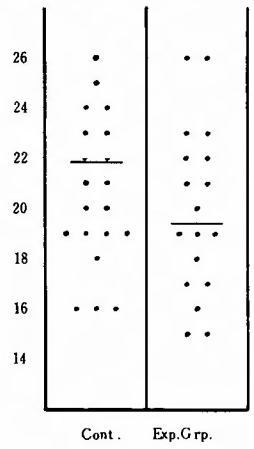
Fig. 5 Survival Days of Animals with Subcutaneous Growth which received Transfer of Spleen Homogenate.



Grp. I Received transfer of spleen homogenate from normal animals.

Grp. II Received transfer of spleen homogenate from tumor-bearing animals.

Fig. 6. Survival Days of Animals with Subcutaneous Growth which received Transfer of Spleen Homogenate from 20-day-aged Tumor-bearing Animals.



Exp. Grp. Received transfer of spleen homogenate from 20-day-aged tumor-bearing animals.

cannot be accepted as significant difference taking considerable fluctuation of transplantability into account (Tab. 6, Fig. 6).

From these studies, it is assumed that spleen homogenate from tumor-bearing animals has inhibitory effect on subcutaneous growth, which, however, is conspicuous when the homogenate is prepared from animals bearing 8 day aged tumor when reticuloendothelial function is in a heightened state and if the homogenate is prepared from animals bearing more aged tumor, inhibitory effect on subcutaneous growth is not observed, suggesting important participation of the reticuloendothelial system in inhibitory effect of spleen homogenate of tumor-bearing animals on subcutaneous growth.

c. Histological findings of subcutaneous tumors which showed a tendency of regression following the injection of spleen homogenate from tumor-bearing animals.

Eleven days after subcutaneous inoculation, when the injection of spleen homogenate is initiated, tumor tissue exists in a mass in the subcutaneous adipose tissue being accompanied by slight appearance of capillaries with findings of slight inflammation around itself (Fig. 15). Fourteen days after the injection of spleen homogenate, although particular change cannot be found in the tumor tissue itself, conspicuous increase in fibrocyts and infiltration of histiocyts were observed, in the surrounding connective tissue, which is accepted as granulation (Fig. 16). Ten days later, an abscess was observed containing a small mass of seemingly necrotizing tumor tissue in it, which is accompanied by granulation formation around it, being surrounded further by proliferation of collagen fibres (Fig. 17). Further 8 days later, tumor cells have completely disappeared showing proliferation of granulation and collagen fibres, which is covered with a crust of necrotized epidermis (Fig. 18). Such healing process as observed is essentially identical with that observed in spontaneous regression and any specific findings were not found.

7. Cytotoxicity of spleen homogenate from tumor-bearing animals.

As observed in the above, it is ascertained that spleen homogenate displays inhibitory effect on subcutaneous tumor growth. Here arises another question whether the inhibitory effect acts immediately upon tumor cells themselves or premises some mechanism of tumor-bearing organism. In order to clarify this problem, inoculation was performed with tumor cells incubated with spleen homogenate from tumor-bearing animals at 37°C for 1 hour, and transplantability, rate of regression and appearance of tumor growth were investigated in 3 groups, each consisted of 20 animals which received subcutaneous inoculations of tumor cells incubated with saline, with spleen homogenate from normal animals and with spleen homogenate from tumor-bearing animals, respectively.

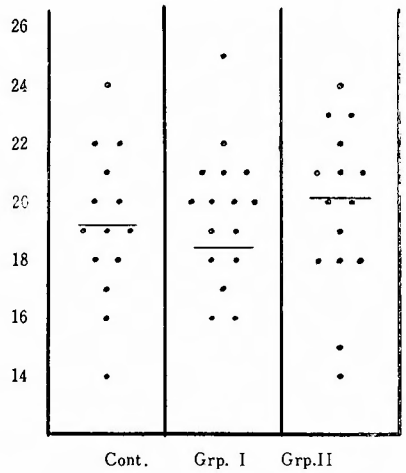
In the control group which was inoculated with tumor cells incubated with saline, out of 20 cases 3 "no takes" and 3 regressions were observed. In the group which received inoculation of tumor cells incubated with spleen homogenate from normal animals 3 cases of "no take" and no case of regression were observed in 20 animals. In the last group which received inoculation of tumor cells incubated with spleen homogenate from tumor-bearing animals, 5 "no takes" and 1 regression were observed in 20 animals.

From these studies, it is impossible to assume that growth of tumor is particularly inhibited in any of these 3 groups (Tab. 7, Fig. 7), and it is considered that spleen homogenate does not display cytotoxicity upon tumor cells at least under the condition of

Tab. 7. Transplantability of Tumor Cells Incubated with Spleen Homogenate from Tumor-bearing Animals.

Medium of Incubation	No. of Inoculation	No. of "No take" (%)	No. of "Take"	No. of Regression (%)
Saline	20	3(15.0)	17	3(17.6)
Spleen homogenate from normal animals	20	3(15.0)	17	0(0)
Spleen homogenate from tumor-bearing animals	20	5(25.0)	15	1(6.7)

Fig. 7. Survival Days of Animals Inoculated with Tumor Cells Incubated with Spleen Homogenate.



Grp. I Inoculation of tumor cells incubated with spleen homogenate from normal animals.

Grp. II Inoculation of tumor cells incubated with spleen homogenate from tumor-bearing animals.

incubation at 37°C for 1 hour. Hereupon, it is strongly suggested that the participation of the reticuloendothelial system is essential for manifestation of inhibitory effect of spleen homogenate from tumor-bearing animals.

8. Influence of spleen homogenate upon reticuloendothelial function of normal animals.

Since the inhibitory effect of spleen homogenate is not displayed by immediate cytotoxicity, it is naturally presumed that the reticuloendothelial system of the hosts should be activated in the direction of antitumoral defence by the transfer of spleen homogenate. Hence, the alteration of reticuloendothelial function caused by transfer of spleen homogenate was studied with congo-red test in normal rats.

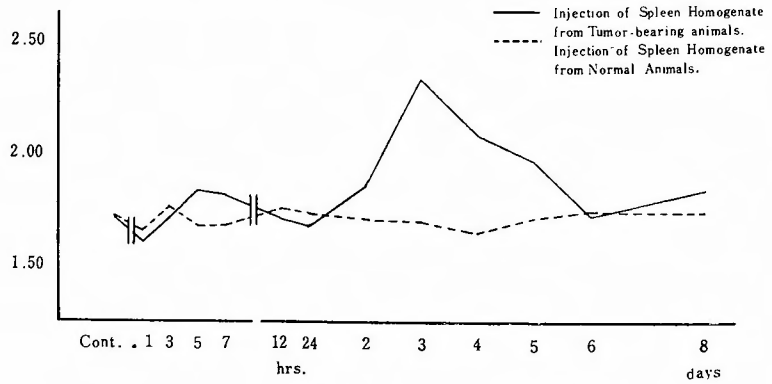
Two groups of normal animals were given subcutaneous injection of 0.5cc of spleen homogenate from normal animals and tumor-bearing animals respectively. In the group which received injection of spleen homogenate from normal animals, congo-red index remained for long around normal level, although slight fluctuation was observed. On the contrary, in the group which received injection of spleen homogenate from tumor-bearing animals, the index showed a slight tendency of increase towards 2 days after the injection and decreased thereafter restoring to normal level 6 days after the injection (Tab. 8, Fig. 8).

9. Reticuloendothelial function of host which showed tumor regression following the injection of spleen homogenate from tumor-bearing animals.

Tab. 8. Fluctuation of Congo-red Index in Normal

Injection	Control	Time after Injection				
		1 hr.	3 hrs.	5 hrs.	7 hrs.	12 hrs.
Spleen Homogenate from Normal Animals	1.72 (1.04-2.57)	1.65 (1.00-2.61)	1.76 (1.14-3.10)	1.68 (1.03-2.12)	1.68 (1.10-2.38)	1.76 (1.00-2.31)
Spleen Homogenate from Tumor-bearing Animals		1.61 (1.01-2.69)	1.71 (1.00-3.04)	1.83 (1.19-2.66)	1.81 (1.04-3.10)	1.71 (1.08-2.50)

Fig. 8. Fluctuation of Congo-red Index in Normal Animals following Transfer of Spleen Homogenate.



Tab. 9. Fluctuation of Reticuloendothelial Function of Animals which showed Tumor Regression following Transfer of Spleen Homogenate from Tumor-bearing Animals.

	Control	Days after Inoculation			
		18	25	32	39
		Days after Transfer of Spleen Homogenate			
		3	10	17	24
No. of Animals	20	10	7	6	6
Spleen Index	0.34 (0.10-0.74)	0.74 (0.33-1.12)	0.77 (0.32-1.00)	0.89 (0.72-1.12)	0.88 (0.65-1.10)
Liver Index	3.8 (3.2-5.6)	4.5 (3.3-5.7)	4.4 (3.6-5.0)	4.5 (3.6-5.2)	4.6 (3.9-5.3)
Splenic Uptake of ^{198}Au-Colloid (%)	0.38 (0.21-0.64)	0.51 (0.27-0.82)	0.51 (0.30-0.90)	0.41 (0.30-0.66)	0.57 (0.40-0.81)
Congo-red Index	1.78 (1.11-2.51)	2.57 (1.72-3.12)	2.36 (1.58-3.15)	2.56 (1.52-3.10)	2.72 (2.21-3.11)

Rats after Injection of Spleen Homogenate.

(Each data represents average of 6 to 10 animals)

Time after Injection						
1 day	2 days	3 days	4 days	5 days	6 days	8 days
1.71 (1.04-2.23)	1.68 (1.10-2.06)	1.70 (1.17-2.04)	1.65 (1.00-2.20)	1.71 (1.01-2.32)	1.74 (1.35-1.99)	1.74 (1.74-1.98)
1.67 (1.09-2.67)	1.85 (1.19-2.26)	2.33 (1.63-3.10)	2.08 (1.10-2.98)	1.97 (1.30-2.57)	1.73 (1.19-2.70)	1.84 (1.13-3.11)

It was studied that reticuloendothelial function is activated when spleen homogenate of tumor-bearing animals is transfered into normal animals. Consequently, reticuloendothelial function was pursued in the animals which showed tumor regression following injection of spleen homogenate from tumor-bearing animals in order to study the attitude of reticuloendothelial function and its participation in the tumor regression caused by transfer of spleen homogenate from tumor-bearing animals.

a. Spleen index.

Spleen index was observed to be 0.74 on the average 3 days after the last injection of the spleen homogenate, which is approximately twice as much enlarged compared with the index of host without treatment in the same stadium. The index remained in the enlarged level thereafter (Tad. 9, Fig. 9, a).

b. Liver index.

Liver index also showed roughly the same tendency, showing enlarged value of 4.5, 3 days after the last injection of the spleen homogenate, which is well maintained thereafter (Tab. 9, Fig. 9, b).

c. Splenic uptake of colloidal radiogold.

Splenic uptake of colloidal radiogold showed markedly increased value of 0.51 per cent 3 days after the last injection of the spleen homogenate, which is followed by little fluctuation (Tab. 9, Fig. 9, c).

d. Congo-red index.

The index was markedly increased to be 2.57, 3 days after the last injection of the spleen homogenate, which was maintained to be 2.36, 10 days after the injection, to be 2.56, 17 days after the injection and to be 2.72, 24 days after the injection, being kept in its increased level (Tab. 9, Fig. 9, d).

These findings were interpreted to suggest that reticuloendothelial function is persistently maintained for long in activated state in cases in which subcutaneous growth inclines to regress following transfer of spleen homogenate from tumor-bearing animals.

10. Influence of reticuloendothelial system blockade on inhibitory effect of spleen homogenate.

a. Inhibitory effect of spleen homogenate on subcutaneous growth in reticuloendothelial system blocked animals.

Tumor of intraperitoneal 7 day growth was subcutaneously inoculated in the rats whose reticuloendothelial system had been blocked by successive injection of india ink for 7 days. Eleven days after the inoculation, injection of spleen homogenate from tumor-

Fig. 9. Fluctuation of Reticuloendothelial Function in Tumor Regression following Transfer of Spleen Homogenate. a. Spleen Index.

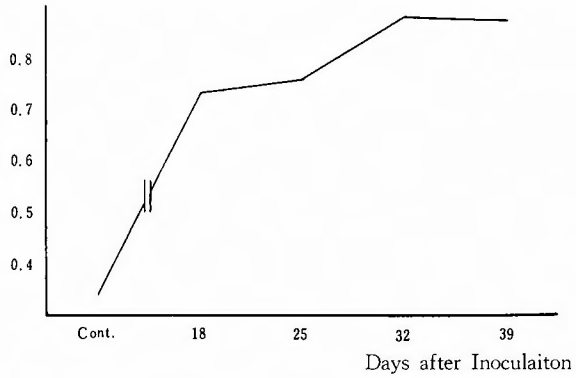


Fig. 9. Fluctuation of Reticuloendothelial Function in Tumor Regression following Transfer of Spleen Homogenate. b. Liver Index.

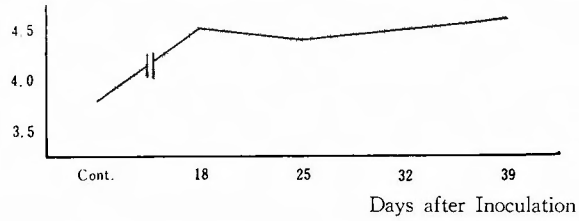


Fig. 9. Fluctuation of Reticuloendothelial Function in Tumor Regression following Transfer of Spleen Homogenate. c. Splenic Uptake of ^{198}Au-colloid

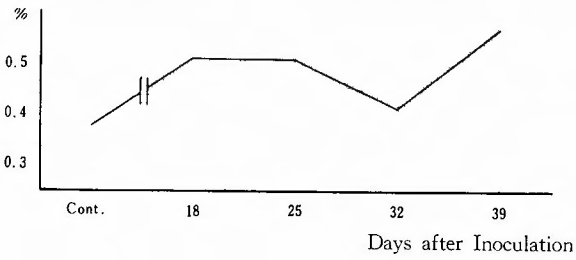
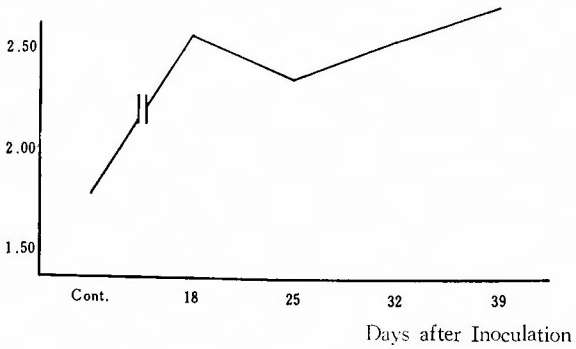


Fig. 9. Fluctuation of Reticuloendothelial Function in Tumor Regression following Transfer of Spleen Homogenate. d. Congo-red Index.



bearing animals was initiated routinely, reticuloendothelial blockade being continued during the treatment of spleen homogenate and it was studied whether or not the tumor regression still occurs even in the hosts whose reticuloendothelial system had been blocked.

In experimental group, out of 20 inoculations, 5 cases of "no take" and no case of regression were observed and in control group, out of 20 inoculations 3 cases of "no take" and 2 regressions were observed showing no significant difference. Concerning survival days, in the former it ranged from 14 to 27 days, 20.6 days on the average and in the latter from 16 to 29 days, 21.4 days on the average, also without any significant difference.

Thus inhibitory effect of spleen homogenate from tumor-bearing animals was not observed in the hosts whose reticuloendothelial system had been blocked with successive intravenous injection of india ink (Tab. 10, Fig. 10).

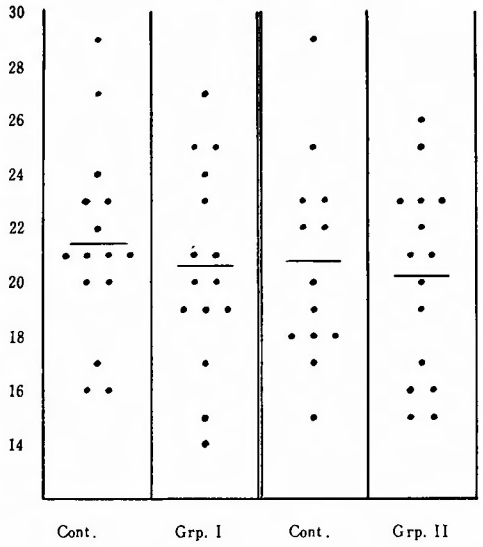
Tab. 10. Influence of Reticuloendothelial Blockade on Inhibitory Effect of Spleen Homogenate.

	No. of Inoculation	No. of "No Take" (%)	No. of "Take"	No. of Regression (%)	No. of Death	Survival Days
Control	20	3(15.0)	17	2(11.8)	15	21.4 (16–29)
Exp. Group I*	20	5(25.0)	15	0(0)	15	20.6 (14–27)
Control	20	2(10.0)	18	5(27.8)	13	20.7 (15–29)
Exp. Group II**	20	2(10.0)	18	3(16.7)	15	20.1 (15–26)

* Group of tumor-bearing animals with blocked reticuloendothelial system which received transfer of spleen homogenate from tumor-bearing animals.

** Group of tumor-bearing animals which received transfer of spleen homogenate from reticuloendothelial blocked tumor-bearing animals.

Fig. 10. Survival Days in Experiment on Influence of Reticuloendothelial Blockade on Inhibitory Effect of Spleen Homogenate.



Grp. I Injection of spleen homogenate from tumor-bearing animals in reticuloendothelial blocked tumor-bearing animals.

Grp. II Injection of spleen homogenate from reticuloendothelial blocked tumor-bearing animals in animals bearing subcutaneous growth.

b. Inhibitory effect of spleen homogenate from tumor-bearing and reticuloendothelial system blocked animals on subcutaneous growth.

This experiment was designed to clarify whether or not the spleen homogenate from tumor-bearing animals, whose reticuloendothelial system is blocked, has an ability to activate the reticuloendothelial function when transfered into other hosts whose reticuloendothelial function is not disturbed. Spleen homogenate in this experiment was prepared from 8 day aged tumor-bearing animals which had received subcutaneous inoculation after preceding reticuloendothelial blockade for 7 days and the blockade had been continued for 8 days further. Thus prepared spleen homogenate was injected in 20 rats bearing 11 day aged subcutaneous growth. Two cases of "no take" and 3 regressions were observed in this group, and in control group of 20 animals, 2 cases of "no take" and 5 regressions were observed showing no particular difference between these 2 groups. Concerning survival days, it ranged from 15 to 26 days, 20.1 days on the average in the former, and in the latter from 15 to 29 days, 20.7 days on the average, showing no difference between both of these groups.

From these studies it is assumed that sound and active function of the reticuloendothelial system of both animals which receive transfer of the spleen homogenate and those which provide spleen homogenate is essential and indispensable for the manifestation of inhibitory effect of spleen homogenate and it is presumed, in addition, that such inhibition is displayed only through active function of the reticuloendothelial system.

IV. DISCUSSION.

It is widely accepted that the reticuloendothelial system is the most important system for various defence mechanism of organism[11,46,61,67,68], and resistance of organism against various invasions, such as infections, irradiation[70], shock[6,18,35,46] and neoplasms[21,39,46,54,57,68,69], largely depends upon the function of the reticuloendothelial system. There are numerous reports that demonstrated that resistance against infections is markedly weakened by reticuloendothelial blockade and production of antibodies is depressed. Clinically, when splenectomy is performed in the adults, no conspicuous disturbance is noticed since defence mechanism is promptly compensated by the liver, bone marrow and other remainders of the reticuloendothelial system in the whole body. On the contrary, it is reported that in children splenectomy often causes overwhelming and fatal infections[34].

Concerning the reticuloendothelial function during tumor development, OLD (1961)[39,40] reported the results of his experiments on subcutaneous growth of Sarcoma 180. According to his report, reticuloendothelial function reaches its peak towards 7 to 12 days after inoculation, then falls gradually thereafter as tumor grows further and restores to normal immediately before death of the hosts. He explained this fluctuation of reticuloendothelial function during tumor development that small amount of tumor cells acts on the reticuloendothelial system as activating, while excessive amount of tumor cells acts on the reticuloendothelial system as destructive. STERN also observed the similar findings in his experiments using Guérin carcinoma. These results, however, may be somewhat different according to the character of tumor used, route of inoculation and so on. HALPERN (1960)[26] reported that when Ehrlich ascites tumor was inoculated intraperitoneally, reticulcendo-

thelial function remained to be normal until 7 th day and fell gradually thereafter, and any alteration of reticuloendothelial function was observed in subcutaneous growth, and on the other hand, when the tumor was inoculated intravenously, the function was slightly increased, reaching its maximum 5 days after inoculation and restoring to normal 12 days after it. Similarly using Ehrlich ascites tumor, ISHIBASHI (1962)[29] reported that decline of reticuloendothelial function is more remarkable in intraperitoneal growth than in subcutaneous growth, and he further reported based upon his experimental and clinical observations that production of antibodies is generally depressed in tumor-bearing organisms; that is, he observed that duration of "take" of heterologous skin graft is prolonged in tumor-bearing animals, occurrence of anaphylaxis with ovalbumin is hindered in tumor-bearing animals, production of antibody against brucella is suppressed in these animals, and production of tetanus antitoxin is lower in cancer patients. In clinical observations, YAMAGATA (1954, 1962)[67,68,69] reported that reticuloendothelial function, as determined by phagocytic activity, is lowered in patients of cancer in the stomach, and in addition the function behaved in parallel with the development of the disease and moreover it was improved when the primary tumor is favorably removed. STERN (1960)[54], SAKAI and OMORI (1962)[46] and others also studied reticuloendothelial function in patients with cancer in the stomach using congo-red test. STERN observed reticuloendothelial hypofunction in 86 per cent and SAKAI and OMORI in 80 per cent, both of them reporting that the degree of hypofunction had correlation to the development of the disease. In the present experiment, in which ascites hepatoma AH 130 was used, phagocytic activity of the reticuloendothelial system reached its peak 8 days after subcutaneous inoculation and most conspicuous splenic enlargement was also observed at this stage. The fact that the reticuloendothelial system in most patients of stomach cancer shows hypofunction is presumably interpreted that it must be considerably late stadium of the whole course of cancer when these patients come to receive the examinations. Accordingly, it is not quite impossible to presume that there might exist, as OLD asserted, a certain stadium in which small amount of tumor cells is activating the reticuloendothelial system.

Although all these experimental and clinical reports on reticuloendothelial function in tumor-bearing organisms do not always come to accordance, there are also numerous attempts to explore participation of the reticuloendothelial system in host resistance, by examining the influence of activating or depressing treatment of this system on tumor establishment and development. It is widely admitted that reticuloendothelial function is easily depressed by various colloidal pigments, colloidal carbon[40,67] or silver[67], thorotrast[13], steroid hormone[43], irradiation[70] and so on, while the function is activated by some substance containing endotoxin[40,61], Zymosan[40], BCG[40], splenic extracts[67], heterologous serum[61], certain polysaccharides, vitamin K, carotin, sodium thiosulfate[67] and so on. IWASE and FUJITA observed that incidence of DAB hepatoma is lowered by long and successive intravenous injection of 1 per cent trypan blue, which causes hyperplasia of the reticuloendothelial system. FUKUYOSHI also reported that antitumoral resistance was promoted and intrahepatic tumor establishment following inoculation from the portal vein was hindered by the same procedure, and on the contrary, host resistance was weakened by 3 per cent lithion-carmin, which caused to block the reticuloendothelial system. BROUWER[13] observed that susceptibi-

lity to transplantable tumor was increased by a single injection of thorotrast of 1 cc. POMEROY[13] observed wide-spread metastases following inoculation in the animals previously treated with cortisone. The same findings were observed in patients with breast cancer by HARTMAN. STERN and WILLHEIM and others reported that growth and metastasis formation of Flexner-Jobling carcinoma and Jensen sarcoma were markedly hindered by administration of carotin. Although these treatments are believed to act on the reticuloendothelial system as activating or depressing and to cause alteration of neoplastic process, it is not that immediate demonstration of such mechanism is disclosed. All these agents manifest sometimes adverse results according to the dosis of administration and even cortisone is not exceptional[20]. SHIMURA's report[31] is accepted to have demonstrated more immediately the significance of the reticuloendothelial system in tumor development, in which he observed prolongation of survival days in the animals inoculated with Ehrlich ascites tumor when liver regeneration is most prosperous after partial hepatectomy. On the other hand, there have been many experiments since early days that tumor development was accelerated by splenectomy[7,37].

As obvious from these results, it is easily accepted that the reticuloendothelial system has important influence upon tumor growth and FUJINAMI[21] insisted that it is always of utmost importance to protect and activate reticuloendothelial function in the aim of promoting general defence at surgery in cancer. It is easily presumed that the spleen which covers one third of the whole reticuloendothelial system of organism plays an important role in antitumoral defence. Actually, during development of experimental tumor outstanding enlargement of this organ is observed[12,33,40,46], it is far difficult to implant tumor cells into spleen parenchyma compared to implantation into other organs around this for instance into the pancreas[7] and this organ is rarely involved in both primary and secondary lesion of malignant neoplasms[12], which is, however, to some extent due to anatomical relationship of the organ. All these facts have attracted interest of many investigators upon this organ.

Splenic enlargement during tumor development was first observed by BRANCATI and others. Here it becomes the question whether this enlargement is due to functional hypertrophy caused by increased production of tumor antibody[12] or certain tumor destructing ferment[11], or due to reactive hypertrophy caused by products of abnormal metabolism of tumor tissue or products of tumor destruction itself. OLD[39,40] observed that such splenic enlargement appears already in the early stadium when the tumor is slightly palpable and in the present experiments also splenic enlargement was most conspicuously observed 8 days after subcutaneous inoculation and as the tumor grew huge and immediately before the death of the animals remarkable atrophy of the organ was observed. Accordingly, it is difficult to assume that the splenic enlargement is caused by products of abnormal metabolism of tumor or tumor destruction. ROFFO[15] observed that splenic enlargement is caused not only by transfer of tumor cells, but caused in normal animals also by blood of tumor-bearing animals. MURATA (1959) also reported the similar findings using Ehrlich ascites tumor and SAKAI observed the existence of substance causing splenic enlargement in urine of cancer patients. Furthermore, according to NAITO[38] this substance fails to cause splenic enlargement when administered in excessive concentration, thence he presumed the

presence of some factor in this substance which causes disturbance and paralysis of splenic response, and he attributed the cause of atrophy of the spleen in the late stadium of experimental tumors to this factor.

It was BORREL and BRIDRÉ[10] who first demonstrated inhibitory effect of spleen on tumors. BRAUNSTEIN[12] investigated this systematically further and reported that susceptibility to transplantable tumor is markedly increased by splenectomy, spleen homogenate frequently shows an effect to inhibit subcutaneous growth and the inhibitory effect is more decisive in spleen homogenate prepared from tumor-bearing animals than that prepared from normal animals. He observed furthermore that spleen homogenate from animals bearing early tumor growth has less effect compared with that from tumor-bearing animals in a stadium of active tumor growth. From these findings of his experiments he concluded that tumor antibody is chiefly produced in the spleen. This inhibitory effect of spleen homogenate of tumor-bearing animals was further studied and ascertained thereafter by OSER and PRIBRAM, APOLANT, BIACH and WELTMANN[7] and others. In the present experiment, inhibitory effect of spleen homogenate was observed when the homogenate was prepared from 8 day aged tumor-bearing animals, when tumor growth is relatively active, while the homogenate from animals bearing 20 day aged huge tumor no longer demonstrated any inhibitory effect. It is interesting to consider these findings with the fluctuation of reticuloendothelial function during tumor development, and it is suggested that manifestation of inhibitory effect of spleen homogenate from tumor-bearing animals premises actively functioning reticuloendothelial system. The finding that the inhibitory effect was not observed both when the homogenate was injected in the tumor-bearing animals with blocked reticuloendothelial system and on the contrary, when the homogenate from tumor-bearing animals with blocked reticuloendothelial system was injected into the tumor-bearing animals with active reticuloendothelial function, and the finding that tumor growth did not undergo any alteration following subcutaneous inoculation of tumor cells incubated with the spleen homogenate, all these findings are accepted to demonstrate that the inhibitory effect of spleen homogenate upon tumor growth does not act as cytotoxic, but is displayed only through active function of the reticuloendothelial system.

It contains very complicated problems and requires further study to clarify whether such inhibitory effect of spleen homogenate from tumor-bearing animals is essentially due to non-specific defence mechanism of the reticuloendothelial system or due to immunological mechanism which premises the presence of tumor antibody. As mentioned in the above, BRAUNSTEIN attributed the cause of antitumoral effect of spleen to the possible increase of antibody production in the organ. It is not impossible to assume that conspicuous increase in plasma cells in the spleen, as observed in the present experiment, supports the presumption of BRAUNSTEIN, since plasma cell is widely thought to be the most important cells of antibody production[3,16,27,65]. It is interesting further that in the present experiment, transplantability of intraperitoneal inoculation was observed to be 100 per cent, while "no take" was observed in considerable frequency in subcutaneous inoculation, and subcutaneous growth often inclined to spontaneous regression and moreover it showed very frequent regression following the transfer of spleen homogenate of tumor-bearing animals. WITEBSKY (1961)[66] pointed out that the antigenicity of tissue or cells is determined by its

environment, and ISHIBASHI (1962)[28,29] postulated that there appears certain difference in antigenicity of tumor and in the degree of stimulation upon antibody producing cells according to the site of tumor growth. In his experiments using Yoshida sarcoma and ascites hepatoma MH 134, he observed a possible establishment of autoimmunity when growth of cancer cells, endowed with organ specificity, is going on apart from their original site, and as a clinical instance of this phenomenon he pointed out occurrence of sudden cessation of tumor growth immediately after an establishment of metastasis. In the present experiment, when tumor was inoculated in the original site of the peritoneal cavity, transplantability was almost absolute without spontaneous regression and tumor growth was irrevocable without being affected by spleen homogenate, whereas once the tumor was inoculated subcutaneously apart from the original site, transplantability became markedly lowered and uncertain, showing frequent spontaneous regression and tumor growth was conspicuously altered by the transfer of the spleen homogenate. These findings are well comprehended by the help of conception of possible establishment of autoimmunity in tumor growth apart from the original site.

In the present experiment it was observed that reticuloendothelial hyperfunction was caused by the transfer of spleen homgenate of tumor-bearing animals, which was maintained relatively long. Consequently, it is presumed that the transfer of the spleen homogenate activates the reticuloendothelial system and promotes the establishment of autoimmunity, and it is anticipated that new approach to clinical application of this phenomenon should be investigated further, together with anti-reticular cytotoxic serum as asserted by BOGOMOLETS[9,58] and SKAPIER[54,58].

V. SUMMARY AND CONCLUSION.

Inhibitory effect of spleen homogenate from tumor-bearing animals on tumor growth was studied using ascites hepatoma AH 130 and results are summarized as follows :

1. Intraperitoneal growth was not influenced by the transfer of spleen homogenate from tumor-bearing animals.

2. In subcutaneous inoculation, "no take" was observed in 22.0 per cent and spontaneous regression in 10.3 per cent, and in 76.3 per cent tumor regression was observed following transfer of spleen homogenate of tumor-bearing animals.

3. Reticuloendothelial function of tumor-bearing animals was most elevated 8 days after subcutaneous inoculation, which declined gradually thereafter in parallel with further development of the tumor.

4. The inhibitory effect of spleen homogenate from tumor-bearing animals does not act as cytotoxic, since there was no significant alteration in transplantability and survival time, even if the inoculation was performed with incubated tumor cells with spleen homogenate from tumor-bearing animals.

5. Reticuloendothelial function is maintained in activated state for long in the animals which showed tendency of tumor regression following transfer of spleen homogenate from tumor-bearing animals.

6. It is assumed that actively functioning reticuloendothelial system is essential for manifestation of inhibitory effect of spleen homogenate and the inhibitory effect is no longer observed if the reticuloendothelial system of both animals providing spleen homo-

genate and those receiving transfer of spleen homogenate is blocked with india ink.

7. In intraperitoneal inoculation, transplantability was absolute and tumor growth was not influenced by the transfer of spleen homogenate of tumor-bearing animals, whereas in subcutaneous inoculation, transplantability is markedly lowered, showing spontaneous regression and tumor regression was observed frequently following the transfer of spleen homogenate of tumor-bearing animals, which strongly suggests that the transfer of spleen homogenate remarkably prompts an establishment of autoimmunity in tumor growth apart from the original site.

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VI. References

1) Adjutantis, G. : Electrophoretic separation on filter paper of the soluble liver-cell protein of the rat using borate buffer. Nature, **173**, 539, 1954.
2) Adler, H., Reimann, F. : Beitrage zur Funktionsprüfung des reticuloendothelialen Systems. Z. exp. Med., **47**, 617, 1925.
*3) Amano, S. et al. : On antibody production of plasma cells. Igaku-soran, **1**, 63, 1945.
4) Amos, D. B., Wakefield, J. D. : Growth of mouse ascites tumor cells in diffusion chambers. I. Studies of growth rate of cells and of the rate of entry of antibody. J. Nat. Canc. Inst., **21**, 657, 1958.
*5) Aoki, H. : On formation of Heinz bodies. Nisshin-igaku, **41**, 230, 1954.
6) Benjamin, W. et al. : Influences of reticuloendothelial system on response to hemorrhage and trauma. RES. Bulletin, **3**, 31, 1957.
7) Biach, P., Weltmann, O. : Über den Wachstumhemmenden Einfluss der Milz auf das Rattensarkom. Wien. Klin. Wschrft., **26**, 1115, 1913.
8) Black, M. M. et al. : Lymph node structure in patients with cancer of the breast. Am. J. Path., **29**, 505, 1953.
9) Bogomolets, A. A. : Cited from the paper of R. Straus. Studies on antireticular cytotoxic serum. I. Introduction and review of the literature. J. Immunol., **54**, 151, 1946.
10) Borrel u. Bridré : Cited from the paper of P. Biach. Über den Wachstumhemmenden Einfluss der Milz auf das Rattensarkom. Wien, Klin. Wschrft., **26**, 1115, 1913.
11) Böhme, D. : Non-specific resistance and the reticuloendothelial system. Tohoku J. Exp. Med., **71**, 121, 1959.
12) Braunstein, A. : Zur Frage der Krebsmetastasen in der Milz. Z. f. Krebsforsch., **32**, 119, 1930.
13) Brouwer, P. : Untersuchungen über das Geschwulstwachstum bei Kaninchen nach Vorbehandlung mit Thorotrast. Beitr. klin. Chir., **168**, 616, 1938.
14) Chalatow, : Zur Frage über die metastatische Geschwulstbildung in der Milz. Virchows Arch., **217**, 140, 1914.
15) Cirio, L., Balestra, G. : Tumori primitivi, tumori metastatici e irradiazione X generale nel topo bianco. Pathologica, **22**, 451, 1930.
16) Coons, A. H. et al. : Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J. Exp. Med., **102**, 49, 1955.
17) Crile, G. Jr. : Factors influencing the spread of cancer. Surg. Gyne. and Obst., **103**, 342, 1956.
18) Davis, H. A. : Pathology of shock in man. Arch. Surg., **41**, 123, 1940.
19) Dunphy, J. E. : Some observations on the natural behavior of cancer in man. New Engl. J. Med, **242**, 167, 1950.
20) Emerson, W. J. et al. : Pathology of primary and recurrent carcinoma of the human breast after administration of steroid hormones. Cancer, **6**, 641, 1953.
*21) Fujinami, S. : Tumor growth and general immunity. Gann, **48**, 340, 1957.
22) Grace, J. T. Jr., Kondo, T. : Investigations of host resistance in cancer patients. Ann. Surg., **148**, 633, 1958.

23) Greene, N. S. H. : The transplantation of tumors to the brains of heterologous species. Canc. Res., **11**, 529. 1951.
24) Greene, N. S. H. : The transplantation of tumors to the brains of heterologous species. Canc. (Philad.) **5**, 24, 1952.
25) Gummel, H., Wittig G., : Über die Notwendigkeit einer Wertbestimmung maligner Tumoren. Chirurg, **10**, 448, 1960.
26) Halpern, B. N. : Reticuloendothelial structure and function. Ronald Press. Co. p. 259. 1960.
*27) Hanaoka, M. : Minute structure and protein synthesis in plasma cells. Nihon-rinsyo, **17**, 638, 1959.
*28) Ishibashi, Y. : Autoimmune therapy of cancer. Surgical Therapy, **6**, 1, 1962.
*29) Ishibashi, Y. et al. : The reticuloendothelial system and tumor-bearing host. Saishin-igaku, **17**, 1102, 1962.
*30) Ishibashi, Y. et al. : Studies on host resistance in cancer. J. J. S. S. (Nihon-Gekagakkai-Zasshi) **61**, 1055, 1960.
*31) Ichikawa, O. : Cytochemistry —The theory and procedures—. p. 109. Honda-shoten. Tokyo. 1953.
32) Jelin, W. et al. : Über den Einfluss der Immunisierungsmethodik blockierter Kaninchen auf die Bildung von Antikörpern. Z. Immunitätforsch. u. exp. Therap., **65**, 440, 1930.
33) Lewin, C., Meidner, S. : Versuche Über die Heilwirkung des Milzgewebes von Tumortieren. Z. f. Krebsforsch., **11**, 364, 1912.
34) Lucas, R. V. Jr., Krivit, W. : Overwhelming infection in children following splenectomy. J. Pediatr., **57**, 185, 1960.
35) McKenna, B., Zweifach, W. : Reticuloendothelial system in relation to drumshock. Am. J. Physiol., **187**, 263, 1956.
36) Moore, G. E., Grace, J. T. : Cancer Immunity. Surg. Gyne. and Obst., **110**, 234, 1960.
*37) Mori, G., Kobayashi, M. : Practice of electrophoresis on filter paper. Nankodo Co. Tokyo. 1960.
*38) Naito, K., Tsuzuki, T. : On splenic enlargement by injection of fresh extract of cancer tissue. VIII. (Studies on cachexia of cancerous diseases.) Nihon-igaku, **15**, 3388, 1944.
39) Old, L. J. et al. : Reticuloendothelial function during experimental tumor growth. Proc. Am. Asso. Canc. Res., **3**, 49, 1959.
40) Old, L J. et al. : The role of reticuloendothelial system in the host reaction to neoplasia. Canc. Res., **21**, 1281, 1961.
41) Pelner, L. : Host-tumor antagonism. IX. The reticuloendothelial system and neoplasia. J. Am. Ger. Soc., **5**, 916, 1957.
42) Peterson, W., Colmers, F. : Anatomische und klinische Untersuchungen über die Magen- und Darmcarzinome. Beitr. zur klin. Chir., **43**, 1, 1904.
43) Pomeroy, T. C. : Studies on the mechanism of cortisone-induced metastases of transplantable mouse tumors. Canc. Res., **14**, 201, 1954.
44) Pool, E. H., Dunlop, R. G. : Cancer cells in the blood stream. Am. J. Ca., **21**, 99, 1934.
45) Roffo, A. H. : Eine biologische Reaktion der Milz, hervorgerufen durch Blut tumorbehafteter Ratten. Z. f. Krebsforsch, **30**, 180, 1930.
*46) Sakai, T., Omori, Y. : Significance of reticuloendothelial system in the field of surgery. Saishin-igaku, **17**, 1133, 1962.
*47) Sakita, S. An experimental study on regression of metastasized lymph nodes following removal of primary tumors. personal communication, not yet published.
*48) Saito, M. : Clinical examinations by the use of electrocolorimeter. Nankodo Co. Tokyo. 1943.
49) Sandberg, A. A. : The frequency of tumor cells in the bone marrow and blood. Cancer, **11**, 1180, 1958.
*50) Sawano, T. : Clinical and experimental studies on the function of spleen and reticuloendothelial system. J. Jap. Med. Univ., **17**, 322, 1950.
*51) Shimura, H. Liver and malignant growth. Clinic and Research, **36**, 1374, 1959.
*52) Shimura, H. : Studies on spontaneous healing of malignant tumor I. The role of reticuloendothelial system in the growth of transplantable sarcoma. Gann, **48**, 338, 1957.
53) Skapier, J. : Therapeutic use of anti-reticular cytotoxic serum (A.C.S.) in Hodgkin's disease. Canc. Res., **7**, 369, 1947.
54) Stern, K. : Studies on reticuloendothelial function in relation to growth process. Ann. N. Y. Acad. Sci., **88**, 253, 1960.
55) Stern, K., Duwelius, A. : Phagocytosis in liver and spleen of rats with Lewis lymphoma. Canc. Res., **20**,

587, 1960.
*56) Sugimoto, M. : Experimental studies on recurrence after palliative removal of cancer. J. J. S. S. (Nihon-Gekagakkai Zasshi), **59**, 91, 1958.
*57) Sunada, T. : Some problems on radical operation from a point of biological character of cancer, with special reference to the significance of splenectomy at operation of cancer in the stomach. Clinic and Research, **36**, 1365, 1959.
58) Straus, R. : Studies on antireticular cytotoxic serum. I. Introduction and review of the literature. J. Immunol., **54**, 151, 1946.
59) Tabern, D. L., Lahr, T. J. : A simplified method for determining radioisotopes in tissues. Science, **119**, 739, 1954.
*60) Takizawa, N., Nakano, K. : Character of less frequently metastasizing cancer and its lymph node change. Jap. J. Canc. Clin., **6**, 451, 1960.
*61) Tanaka, S. et al. : Cancer chemotherapeutics and reticuloendothelial system. J. Clin. Digest. Diseas., **4**, 571, 1962.
62) Toolan, H. W. : Conditioning of the host. J. Nat. Canc. Inst., 14, 745, 1953.
63) Towbin, A. : The heterologous transplantation of human tumors. Canc. Res., **11**, 286, 1951.
64) Watne, A. L. et al. : Modification of host resistance to malignant cell growth. Arch. Surg., **83**, 478, 1961.
65) White, R. G. : Observations on the formation and nature of Russell bodies. Brit. J. Exp. Path., **35**, 365, 1954.
66) Witebsky, E. : The question on self-recognition by the host and problems of autoantibodies and their specificity. Canc. Res., **21**, 1216, 1961.
*67) Yamagata, S. : Reticuloendothelial system and liver function. Igaku-shoin. Tokyo. 1954.
*68) Yamagata, S. et al. : Reticuloendothelial function in internal diseases. Saishin-igaku, **17**, 1119, 1962.
*69) Yamagata, S. : Reticuloendothelial system and clinics. Proc. Jap. Soc. RES., 2, 2, 1962.
*70) Yamamoto, M. : On the relationship between irradiation injury and reticuloendothelial system. Saishin-igaku, **17**, 1111, 1962. (* in Japanese)

和 文 抄 録

担癌宿主の抗腫瘍性に於ける網内系の意義に関する実験的研究

—— 特に脾の抗腫瘍性について ——

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悪性腫瘍は多くの場合，一方的に増悪する如く思われるが，詳細に観察するとその経過は可成り不規則であり，無処置の癌が10数年も極めて緩慢に発育した例が報告され，又姑息的手術の後の無症状期間が数10年にも及んだ症例も報告されている．逆に根治的に手術されたと考えられる症例においても術後短期間内に再発，転移を来す場合があり，腫瘍の発育は何らかの形で宿主の網内系機能による影響を蒙むつていることが推定されている．一方網内系の最も重要な臓器である脾は原発性，続発性腫瘍に侵されることが極めて稀であり，実験腫瘍の経過中に著明な脾腫大が観察されており，これらの事実はこの臓器が特異な抗腫瘍性を有することを示唆するものとして注目され，脾の抗腫瘍性を実験的に証明した報告は古くから夥しい数に上つている．

著者は脾の抗腫瘍性を腹水肝癌 AH130 を用いて追試し，更にその発現機序について検討を加えて次の結果を得た．

1) 腹水肝癌 AH130 の腹腔内腫瘍は担癌動物脾 Homogenate によつてその発育が影響を受けることはない．

2) 皮下移植では 22.0% に移植陰性が認められ，10.3%に自然退縮が認められた．担癌動物脾 Homogenate の皮下注射により，76.3%に腫瘍の退縮が観察された．

3) 担癌動物の網内系機能は移植後8日目に最も昂進し，以後漸次低下する．

4) 担癌動物脾 Homogenate と腫瘍細胞を incubate したものを皮下移植しても移植率，生存日数等に変化はなく，担癌動物脾 Homogenate は腫瘍細胞に対して cytotoxic に作用するのではないと考えられる．

5) 担癌動物脾 Homogenate 移入によつて腫瘍退縮の傾向を示す宿主の網内系機能は長く亢進状態を維持することが観察された．

6) 脾 Homogenate を提供する担癌動物の網内系が塡塞されても，又脾 Homogenate の移入を受ける担癌動物の網内系が塡塞されても担癌動物脾 Homogenate の抗腫瘍性は観察されず，この抗腫瘍性の発現には活潑な網内系機能の存在が必須であると考えられる．

7) 腹腔内移植では移植率は 100 % であり，自然退縮もなく，担癌動物脾 Homogenate によつてもその発育が影響されなかつたのに反し，皮下移植腫瘍では移植率は低下し，自然退縮が屢々見られ，担癌動物脾によつて高率に腫瘍退縮が見られた．このことは腫瘍の異所的発育における自家免疫の成立を強く暗示するものと考えられ，担癌動物脾 Homogenate の移入によつてこの自家免疫の成立が促進助長されるものと考える．

（尙 本論文の要旨は第19回，第20回癌学会総会において発表した．）